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Colorado Univ. at Boulder

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SORPTION PROPERTIES OF MODEL COMPOUNDS ON C_{18} ADSORBENTS

by

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16. ABSTRACT

The bonded silica adsorbent Bondapak-C_{18}® was evaluated for removing organic matter from secondary sewage effluents and from solutions of pure organic compounds. The adsorbent is hydrophobic and its behavior with water samples may be erratic unless first wet with a solvent. However, water- ethanol mixtures behaved reproducibly and gave linear adsorption isotherms. Part of the effort was aimed at developing liquid-chromatographic methods of analysis for organic constituents of wastewater. For this work microparticulate adsorbents were used in combination with a Sep-Pak precolumn for rapid trace enrichment. Identification of the many chromatographic peaks was hampered by incomplete resolution. The chromatograms, however, were reproducible permitting comparisons of different effluents and tertiary treatment processes.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report provides the results obtained for the evaluation of C_{18} adsorbents for the isolation and concentration of model compounds for recovering trace organic materials from waste water. Evaluations of synthetic adsorbents are necessary in determining the best available methods for the preparation of representative concentrates of waterborne organic contaminants for *in vivo* and *in vitro* biological testing.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

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ABSTRACT

The bonded silica adsorbent "Bondapak-C_{18}" (Waters Associates) has been evaluated for removing organic matter from secondary sewage effluents and from solutions of pure organic compounds. The adsorbent is hydrophobic and will not function effectively in water unless it is first wet with methanol or another organic solvent; therefore, its behaviour in water may be erratic. In water-methanol mixtures, on the other hand, it behaves reproducibly and gives linear absorption isotherms. Advantages of "Bondapak-C_{18}", compared with microparticulate adsorbents, include greater chemical robustness and tolerance to regeneration, lower flow resistance and lower cost. We have used it to remove organic compounds from water and as a support in low-resolution chromatographic analysis.

Part of our effort was aimed at developing liquid-chromatographic methods of analysis for organic constituents of wastewater. For this purpose microparticulate adsorbents were used and "Sep-Paks" (Waters Associates) were used for rapid trace enrichment. Identification of the many chromatographic peaks is difficult, but the chromatograms are reproducible and allow different effluents to be compared and tertiary treatment processes to be evaluated.

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SECTION 1

INTRODUCTION

The research here described is a continuation of that reported in March 1979: EPA report No. 600/1-79-014; "Chemistry and Cytotoxicity of Renovated Wastewater. In that report a method of analysis of filtered secondary sewage effluents was described that used selective adsorption and desorption of the less polar organic constituents and yielded a number of fractions of successively decreasing polarity. These fractions were tested for toxicity by a micro-scale cellular technique; some tests of mutagenic action were made, and attempts were made to resolve the fractions into individual, identifiable chemical compounds.

The material used to adsorb organic materials from treated wastewater, and to fractionate them by selective desorption with water and methanol, was, in most of the tests, "Bondapak-C_{18}-Porasil B", or simply "Bondapak-C_{18}", produced and sold by Waters Associates for liquid chromatography. This adsorbent was chosen because, when packed into a column, it retained a reasonable proportion of the organic material, about one-half of the total organic carbon, and released nearly all of it when water-methanol mixtures were passed through the column. The usual procedure was to use a solvent gradient, a flowing mixed solvent whose methanol:water ratio was increased continuously with time, so that one began with pure water and ended with pure methanol. The passage of organic material out of the column was continuously monitored by absorption of ultraviolet light. After the UV absorption returned to baseline, the column was restored to its original condition by flushing with pure water. It was then ready to adsorb organic compounds from a fresh quantity of wastewater. The sorption-desorption cycle could be repeated many times before the Bondapak-C_{18} lost its capacity. In this respect, Bondapak-C_{18} was superior to organic polymer absorbents like Amberlite XAD-2, which absorb the brown humic constituents of wastewater very strongly, but release them only incompletely and slowly, so that the sorbent became fouled and choked.

In a typical procedure, a stainlesss steel tube, 50 cm long by 1.0 cm internal diameter, was packed with Bondapak C_{-18}. Up to 10 liters of filtered secondary sewage effluent, containing 15 mg dissolved organic carbon per liter, were pumped through the column under moderate pressure (about 100-200 psi). Then the column was flushed with distilled water, and a water-to-methanol gradient applied over 30 minutes, followed by pure methanol for 20 minutes. Flow rates were of the order 5 mL/min. An ultraviolet absorbance detector (variable or fixed wavelength) was placed at the column exit to monitor the effluent; see our 1979 report. Appropriate fractions of the liquid flowing from the column were collected and concentrated to small volume by vacuum evaporation.

The main purpose of the continued investigation, described in this report, was to study in detail the adsorptive properties of Bondapak-C_{18}. A secondary purpose was to see how this material and others like it could be applied to the chromatographic analysis of the organic compounds in treated wastewater.

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SECTION 2

CONCLUSIONS

The main part of this work was a study of the adsorptive properties, selectivity and elution orders on Bondapak-C_{18}, a bonded adsorbent whose characteristics are very like those of the familiar microparticulate bonded phases that are widely used in high-performance liquid chromatography. Physically, its particle size is larger, 37-75 microns compared to the particles of 10 microns and less used in high-performance liquid chromatography, and the particles are less uniform. Therefore, it gives fewer theoretical-plates when packed in columns; theoretical-plate heights of Bondapak-C_{18} range from 0.5 to 1.0 mm, depending on the compound, the solvent and the flow rate, contrasted with 0.05 mm and less for 10-micron particles. The main advantage of Bondapak-C_{18} for wastewater analysis and the preparation of fractions for toxicity testing is that it is more robust and less prone to fouling by the humic substances. Back pressures are much less than with 10-micron particles, and the material is considerably less expensive.

Using Bondapak-C_{18} to absorb organic compounds from water, one must recognize that it is hydrophobic and not wetted by water. It must first be thoroughly wetted with methanol or another water-miscible solvent; then, when the greater part of this solvent has been washed out, the absorbent can be used to take up organic compounds from water. Its behaviour towards purely aqueous solutions depends strongly on the small amounts of methanol sticking to it, and inevitably the behaviour is irreproducible, making it pointless to try to characterize it by exact physical tests. In mixed water-methanol solvents, however, its behaviour is reproducible and similar to that of micro particulate C_{18} bonded packings, except for the lower plate numbers.

This report presents absorption and retention volumes for many compounds, including substances on the list of Consensus Voluntary Reference Compounds, (Lawrence H. Keith, Analysis of Organic Water Pollutants 13, 1469-1471 (1979)), over a range of methanol: water compositions. From these data the retention volumes in pure water can be roughly estimated. The salts normally present in natural waters do not appreciably affect retention, nor do the humic substances. Though the partition ratios between absorbent and solvent vary greatly from compound to compound, the capacity of the absorbent to hold organic compounds is quite high, of the order 10 milligrams organic carbon per gram of absorbent.

In the second part of this work we explored the use of Bondapak-C_{18} and of microparticulate bonded absorbents for fractionating and analyzing organic compounds in water, including the humic compounds. A preliminary report is given on a method of high-resolution liquid chromatography that uses Sep-Paks, small disposable cartridges packed with a microparticulate bonded adsorbent, to absorb the less polar organic compounds from water and to separate them from accompanying humic material. Chromatograms are complex but reproducible, and distinguish between wastewaters from different sources.

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SECTION 3

RECOMMENDATIONS

Two practical purposes remain to be fully realized: one is the separation of the dissolved organic material in wastewater and treated sewage into well-characterized fractions, with a view to toxicity testing, and the other is the chemical analysis and recognition of specific organic compounds, including nonvolatile compounds, by high-resolution liquid chromatography.

"Bondapak-C_{18}" adsorbs relatively hydrophobic compounds, including caffeine, phenols and chlorophenols, most pesticides, plasticizers and polycyclic aromatic hydrocarbons. After adsorption they are stripped off the column by methanol or water-methanol mixtures containing a high proportion of methanol. Water-soluble compounds like sugars and amino acids, polycarboxylic and hydrocarboxylic acids and urea are not adsorbed. A big analytical problem, which we have not satisfactorily resolved, is presented by the humic and fulvic acids, ill-defined substances of high molecular weight, that are partly adsorbed and partly not adsorbed.

We made a very few tests with other adsorbents, notably the anion-exchange resins, Duolite A-7 and Bio-Rad MP-1. These resins remove organic compounds from water that are not removed by Bondapak-C_{18}. They are harder to regenerate than Bondapak-C_{18} and can be used for only a few adsorption-desorption cycles, but one can visualize a separation scheme in which the wastewater (adjusted to a suitable pH) is passed first through Bondapak-C_{18}, then through Duolite A-7, then through Bio-Rad MP-1. Stripping each sorbent in turn one would obtain three fractions plus the fourth fraction that passed all three of the sorbents. Further discrimination could be obtained by adjusting pH and using different regenerants. I strongly recommend that work be done along these lines.

Separation of "difficult volatile" organic compounds by steam distillation is a process that merits more study.

The ultimate challenge, to a chemist, is the identification and measurement of as many as possible of the organic compounds that are present in treated sewage. We advanced significantly toward this goal through "Sep-Pak" trace enrichment and high-resolution liquid chromatography, as described in the latter part of our report. I recommend that these efforts be continued, with emphasis on selective methods of chromatographic detection, particularly electrochemical, which distinguish oxidizable compounds like phenols and benzidines. Electrochemical techniques are now possible that can be used with gradient elution, which the older techniques could not. The method we studied (Sec. 5, Part 4) looks at the hydrophobic organic constituents, rather than the hydrophilic, but it is the hydrophobic compounds that are generally toxic.

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SECTION 4

ADSORPTIVE PROPERTIES OF BONDAPAK-C_{18}

1. The adsorbent.

Bondapak-C_{18} is a bonded-phase adsorbent designed for large-scale or preparative-scale liquid chromatography. It consists of irregular particles of superficially porous silica, size range 37-75 micrometers, to whose surface is chemically bonded a hydrocarbon layer, equivalent to a coating of paraffin one molecule thick. The molecular fragments bound to silica are octadecyl groups, $C_{18}H_{37}$; hence the name "Bondapak-C_{18}".

Adsorbents like this are widely used in liquid chromatography today. For high-performance liquid chromatography (called "HPLC") very small and uniform particles, diameter 10 micrometers or less, are used, and they are "totally porous", that is, they are penetrated by internal channels and have a large effective surface area. Ideally, every silicon atom on this surface carries a carbon chain, if not $C_{18}H_{37}$, then CH_3 groups used for "capping". The idea is to avoid leaving free Si-OH groups that would otherwise exist at the surface of silica. It is impossible to avoid Si-OH groups entirely, however, and we may consider that a part of the surface of Bondapak-C_{18} consists of Si-OH groups. They change the adsorptive properties, making the particles more prone to adsorb polar compounds and even ionic compounds.

Chemically, the coarse Bondapak-C_{18} closely resembles fine-particle bonded-phase adsorbents like Micro Bondapak-C_{18} (Waters Associates), Partisil ODS (Whatman), Zorbax ODS (Dupont), etc. Physically, it is more suitable for treating large volumes of treated wastewater, because it offers less resistance to flow and is not so easily fouled by humic compounds. These compounds of ill-defined structure and high molecular weight account for at least half of the dissolved organic carbon in secondary-treated sewage.

We noted that Bondapak-C_{18} released "nearly all" of the organic material that it adsorbs from wastewater. A little of this material is irreversibly adsorbed, however, and after long use, the adsorbent, which is originally white, turns black and loses much of its adsorptive capacity. Washing the column with tetrahydrofuran and methylene chloride extracted a little waxy solid but left the adsorbent black and did not restore its original capacity. Data to show the capacity loss are given in Sec. 4, Part 2c.

The surface of Bondapak-C_{18} is hydrophobic; it is like a film of paraffin. Therefore, fresh Bondapak-C_{18} floats on water and is not wetted by it; a column packed with the powder will absorb little or no organic material from water that is pumped through it. To make it absorb organic material the Bondapak-C_{18} must first be wetted with methanol or other organic liquid, like ethanol or acetonitrile. Once wetted with methanol, the adsorbent will make contact with water and will keep its ability to adsorb from water for a long time, presumably because it retains just enough methanol to keep it hydrophilic. Eventually the adsorbed methanol is washed out and the adsorbent loses

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its ability to make contact with water. Data on this phenomenon are presented in Sec. 4, Part 3b.

Regarding chemical selectivity, the partition of pure organic compounds between the adsorbent and a solvent (water or methanol-water mixtures) and the order in which a series of compounds is adsorbed and released, there is little difference between Bondapak-C_{18} and the fine-particle C_{18} bonded phases used in chromatography, and as far as we can see, elution orders found on high performance analytical bonded phase columns are the same as those on Bondapak-C_{18} columns.

2. Studies with treated wastewater.

These studies were made with the effluent from the Metro Denver Sewage Treatment Plant, York Street, taken just before the treated, chlorinated effluent is discharged into the South Platte River. This effluent contains 10-20 ppm dissolved organic carbon, the actual content varying from day to day. Portions of effluent were filtered under suction through glass fiber filter papers (Whatman GF-A and GF-F, porosities 2.0 and 0.7 microns) before use. The pH after filtration (with accompanying loss of carbon dioxide) was about 8; for some tests the pH was lowered to 4 by adding nitric acid.

Columns consisted of stainless steel tubing with appropriate end fittings. They were packed dry with Bondapak-C_{18}, using a vibrator. Dry packing is recommended by the manufacturer. It is easier than slurry packing (which was also used) and gives equal performance. Pure methanol was pumped through the packed columns to displace air and render the packing hydrophilic (see above). Columns of different lengths and diameters were used; the widest were 1.0 cm ID; most were 0.45 cm ID. The wall thickness was 1 mm; end fittings were of the standard "zero dead volume" type with stainless steel frits. Back pressures were of the order of 100-500 psi and increased with use.

Total organic carbon was measured by a Beckman Model 915B analyzer, which converts the carbon to carbon dioxide and measures its infrared absorbance. On any given water sample, the reproducibility of repeated tests was 0.5 ppm or better.

2a. Speed of adsorption. It seems that adsorption is fast enough that equilibrium was reached in our experiments since pumping the water through the column a second time did not reduce the TOC (total organic carbon) or the ultraviolet absorbance any more than passing it a single time. This test was made on our smallest column, 10 cm x 0.45 cm, at a flow rate of 1.5 mL/min (linear flow rate 10 cm/min).

2b. TOC reduction and volume passed; capacity, pH effect. Figure 1 shows results of tests with two Bondapak-C_{18} columns, one 10 cm x 0.45 cm, the other 10 cm x 1.0 cm. Filtered sewage effluent was pumped at a rate that was as nearly constant as possible, but the Madden diaphragm pump used for this purpose did not allow close control of the flow rate. The erratic rise and fall of the dissolved organic carbon may be attributed to uneven flow. The effect of pH is clearly seen. Sorption is more effective at lower pH. We have noted this effect many times. Some of the organic matter is acidic (mainly humic acids) and the undissociated acids are more strongly retained than the acid anions.

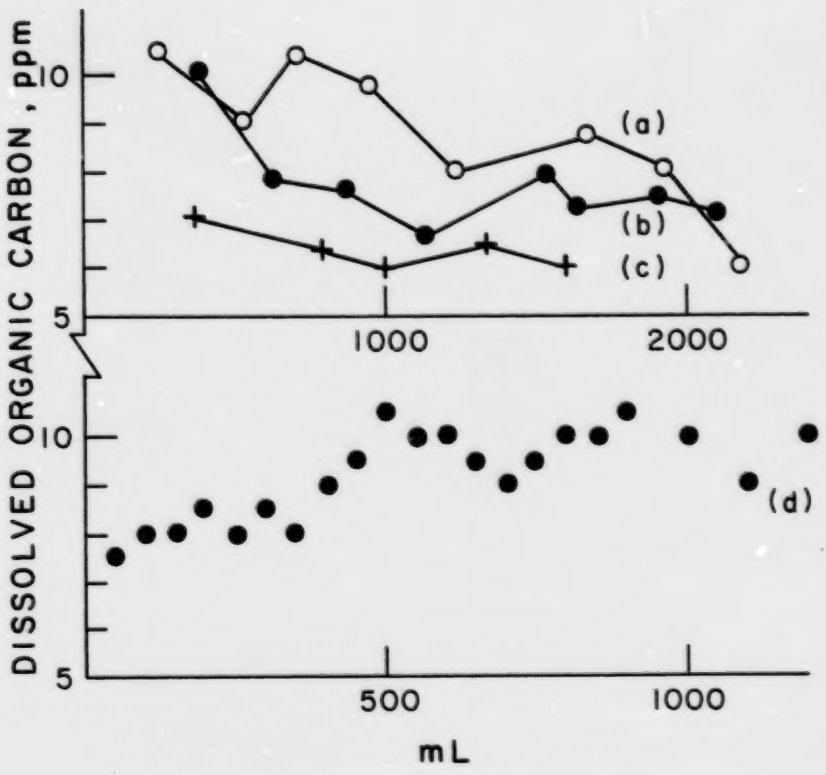

FIGURE 1. DISSOLVED ORGANIC CARBON IN EFFLUENT FROM BONDAPAK-C_{18}.

(a) and (c), column 1 cm x 10 cm; influent, 19.5 ppm carbon; (a), ph 7.2; (c), pH 3.5. Curve (b), column 1 cm x 10 cm; influent, 17 ppm carbon; pH 7.5. Curve (b) was taken a month after (a) and (c) but with the same packed column. Flow rate was about 5 mL/min but could not be controlled accurately. Points (d), column 0.45 cm x 10 cm; influent, 14 ppm carbon, pH 7.5; flow rate, 1.5 mL/min.

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With the larger column the TOC falls as more liquid is passed. At the very beginning of a run the TOC is abnormally high due to methanol that is washed out of the adsorbent, but this effect only lasts for 100 mL at most. The fall in TOC seen here must be due to another effect. Perhaps the humic acid adsorbed on the Bondapak enhances the adsorption of other organic compounds. With the small column, however, the TOC rises slowly, that is, sorption becomes less efficient as time goes on. This is a more understandable effect, for the column must eventually become saturated with adsorbed material. Curve (d), Fig. 1, shows that the column had adsorbed 6 mg of organic carbon during the run. The dry weight of Bondapak in this column was 0.7 gram.

2c. Old and new Bondapak; comparison with other sorbents. Table 1 compares a freshly packed column of Bondapak with one that had been in use for two years and had passed over 100 liters of wastewater; linear flow rates through both columns were the same. Clearly the new Bondapak is a better adsorbent than the old, but old Bondapak is on the whole more effective than the acrylic polymer Amberlite XAD-8, an adsorbent used to collect humic and fulvic acids from natural water.

TABLE 1

REMOVAL OF ORGANIC CARBON FROM WASTEWATER BY DIFFERENT ADSORBENTS

Adsorbent	pH	Dissolved Carbon, ppm influent	effluent	Carbon reduction, percent
New Bondapak,	6.1	18.5	7.6, 7.2	60
0/45 cm x 25 cm	4.5	18.5	7.2	62
Old Bondapak,	8.0	15.2	11.2, 10.5	29
1.0 cm x 50 cm	4.0	15.2	10.8, 9.8	32
XAD-8,	8.0	15.2	12.8	16
0.8 cm x 15 cm	7.5	14.8	12.2, 13.2	14
	4.0	14.8	10.9	26
XAD-8,	7.0	16.1*	13.4*	17
1.0 cm x 20 cm	4.0	16.1*	10.8*	33
XAD-2,	7.0	16.1*	10.4*	35
1.0 cm x 20 cm	4.0	16.1*	6.2*	62

NOTES:

(a) *averages of 4 measurements each

(b) Linear flow rates for old and new Bondapak were both 6.4 cm/min; for XAD resins, flow rates were 1.3-1.5 cm/min.

Additional tests were made with three adsorbents: (a) a custom-made polymer of 2-methyl-5-vinylpyridine (Bio-Rad Laboratories); (b) MP-1, a macroporous strong-base anion exchange resin (Bio-Rad Laboratories); (c) Duolite A7, a weak-base anion-exchange resin (Diamond-Shamrock Co.). They were packed into glass columns, 1 cm ID, to give beds 3 cm long (a and b) or 5 cm long (c). They were tested to see what they would adsorb from secondary treated sewage that had already been passed through Bondapak-

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C_{18}. The hope was that they would remove hydrophilic compounds, including anions of organic acids such as humate ions. They did remove such compounds, but adsorption by the first two materials was irreversible; we could not regenerate these materials.

(a) Polyvinylpyridine. First, 50 mL sewage effluent, pH 7, was passed; the TOC was reduced from 11 ppm to 3 ppm. The column was washed with 0.1M acid followed by 0.1M sodium hydroxide and distilled water; another 50-ml portion of sewage effluent was passed. At pH 7 the TOC was reduced from 11 ppm to only 10 ppm, but at pH 4, it was reduced to 4 ppm. After a second regeneration cycle the TOC was hardly reduced at all, even at pH 4.

(b) Macroporous MP-1. First, 100 mL sewage effluent, pH 7, was passed; the TOC was reduced from 11.5 ppm to 2.0 ppm. The column was regenerated in the same way as was polyvinylpyridine. A second 100 mL of treated sewage effluent, pH 4, had its TOC reduced from 11.5 to 2.5 ppm. Following a second regeration, 150 ml of treated sewage effluent, pH 4, was passed; its TOC was 2.0 ppm at first, rising to 3.5 ppm near the end of the run.

When this bed was stripped with 0.1M hydrochloric acid a very sharp brown-red band was seen to form and move down the column. It yielded 0.65 milligram of organic carbon (by TOC measurements).

(c) Duolite A7. First, 1.7 liters of treated sewage effluent, pH 7, TOC 10.5 ppm, were passed. The TOC of the effluent ranged from 6.5 to 8.0 ppm (readings were taken every 100 mL) averaging 7.0-7.5. Then the column was regerated by passing 1.5M NaOH, water, 2M HCl, water methanol, water, 1.5M NaOH and water; then another 1.5 liters of treated sewage effluent were passed. The TOC rose gradually from 6.0 ppm to 7.0 ppm, fluctuating slightly; samples were analyzed every 100 mL. The carbon recovered on the second regeration (measured as TOC) totalled 2.6 mg; the carbon adsorbed during the second run was 5.2 mg.

It seems that carbon is accumulating in the Duolite column and is not completely removed by regeneration; however, the use of Duolite A7 has possibilities for selective collection of organic material from treated sewage. Summarizing these tests:

Secondary-treated Denver sewage effluent, containing 19.5 - 17.0 ppm TOC (two portions collected on different days) was passed through Bondapak-C_{18}; its TOC fell to 10.5 ppm. Passing this water through Duolite A7 reduced the TOC further to 7.0 ppm. A further reduction to 2.3 ppm could be accomplished by passing through MP-1.

3. Studies with pure compounds.

3a. Experimental; results with benzophenone. These tests were made with columns of Bondapak-C_{18}, 0.45 cm ID and 10-15 cm long. Methanol-water mixtures were pumped through them under pressure, using a Waters pump, model 6000-A or 45; an ultraviolet absorbance detector operated at 254 nm was connected to the column exit. Methanol-water mixtures were used, rather than pure water, because benzophenone is almost insoluble in pure water; moreover, retention times in pure water are extremely large. Compositions of these mixtures are expressed by volume; "40% methanol" means a solvent made by mixing 40 parts of methanol (by volume) with 60 parts of water. Two kinds of tests were made: breakthrough, in which solutions of the organic compounds were pumped through the column until the compounds "broke through" the column and emerged

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at the same concentration level as they went in, and elution, in which small portions (0.1 ml or less) of solution were injected into a flowing stream of solvent, and the compounds were detected as a peak in ultraviolet absorbance when they emerged. "Elution" is the normal mode used in chromatographic analysis, but "breakthrough" and its reverse, "stripping", provides a better evaluation of the behavior of the adsorbent in retaining trace organic compounds from water. Further, "breakthrough" allows one to study the effect of concentration and column loading.

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Curves for breakthrough, stripping and elution of benzophenone in 50% methanol are shown in Figure 2. They are schematic curves summarizing many records on a strip-chart recorder. The steepness of the adsorption-desorption fronts may be expressed through theoretical plate numbers; these numbers ranged from 115 to 165 for a 10 cm column, depending on flow rate, and the curves were symmetrical. Table 2 gives experimental values for V_{ads}, V_{des} and V_{el}; it is seen that these volumes are equal within 1%. We infer that the adsorption of benzophenone on Bondapak-C_{18} follows a linear isotherm; that is, the ratio (concentration on Bondapak):(concentration in solution) is constant and independent of the concentrations themselves. This statement was confirmed by other tests in which the breakthrough volume, V_{ads}, for benzophenone dissolved in 40% methanol was measured over a 1000-fold concentration range. For a column 0.45 cm x 10.5 cm containing 0.92 gram of Bondapak, these volumes were 60 mL at 1 ppm, 54 mL at 100 ppm and 55 mL at 1000 ppm. Results at flow rates 0.5 and 1.0 mL/min were the same. The total quantity adsorbed at 1000 ppm was 55 mg, or 6% the weight of the packing.

If we can assume the isotherm to be linear, even at lowest concentrations, we can predict that the breakthrough volume, V_{ads}, is the same at the very low concentrations prevailing in treated sewage effluents and in polluted waters as it is at the concentrations used in this work. Additional data which justify the assumption of a linear isotherm and show the close fit of experimental and theoretical curves are given in Appendix I- Figure 17. Appendix I also gives full experimental details.

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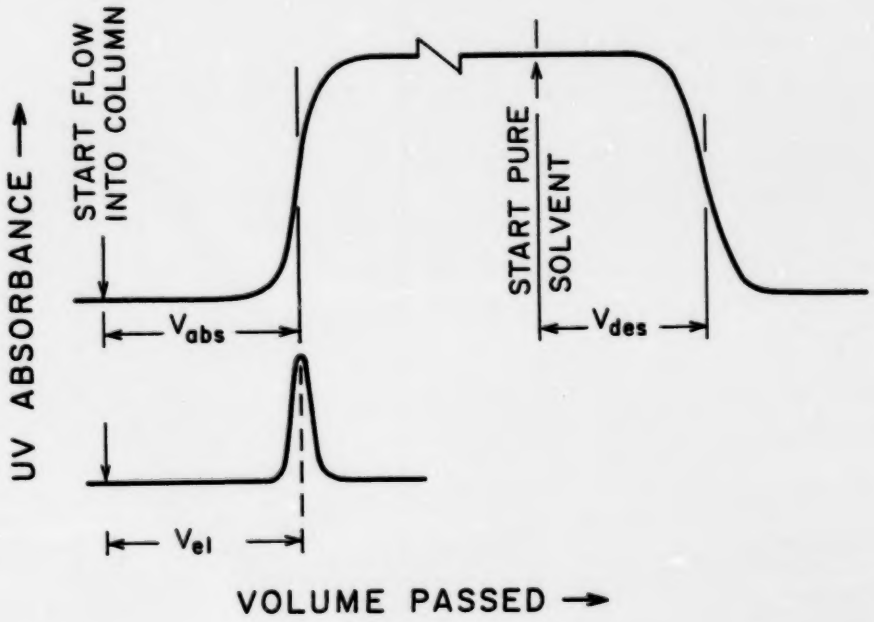

FIGURE 2. ADSORPTION AND DESORPTION OF BENZOPHENONE ON BONDAPAK-C_{18} FROM 50% METHANOL

These are schematic curves drawn from many strip-chart records. Note the symmetrical shape. Theoretical-plate numbers calculated from the curve profiles were 115 for flow rate 1.0 mL/min, 165 for flow rate 0.5 mL/min.

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TABLE 2

ADSORPTION AND DESORPTION VOLUMES OF BENZOPHENONE ON BONDAPAK-C_{18} FROM SOLUTIONS IN 50% METHANOL (v/v)

Benzophenone concentration, ppm:	0.5		2.5	5.0	6.5
Flow rate, mL/min:	1.0	0.5	0.5	0.5	0.5
V_{ads}, mL:	9.6	9.9	9.9, 9.9 10.2, 9.0	9.9	9.9, 9.8 9.8
V_{des}, mL:	9.6	9.9	9.9, 9.9 9.8, 9.8	9.8	9.8, 9.8 9.8

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NOTES:
(a) column dimensions, 0.45 cm x 10.5 cm
(b) theoretical-plate numbers, from adsorption and desorption profiles, 115 (1 mL/min), 165 (0.5 mL/min); see Appendix I for calculation method.
(c) elution volumes, V_{el}, for injections of benzophenone varying between 0.2 and 5.0 micrograms, were 10.0 $\pm$ 0.05 mL (mean of 14 measurements).

The effect of salts on the retention of benzophenone and some other compounds was also studied. EPA had requested that we use the following solution to simulate and average city water:

anhydrous $CaSO_4$ (120 mg/L); $NaHCO_3$ (70 mg/L); $CaCl_2 \cdot 2H_2O$ (47 mg/L), plus nitric acid to give pH 7.5

In some experiments methanol was mixed with the "simulated city water", in others it was mixed with pure water. For compounds that could be eluted in a reasonable time by water alone, pure water was compared with "simulated city water". Results obtained with benzophenone, caffeine, cinnamic acid and furfural are shown in Table 3. They show that there is no detectable difference between pure water and "simulated city water". With furfural, humic acid (J.T. Baker) was added at a concentration like that in treated sewage; no effect of humic acid on the retention of this compound was found.

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TABLE 3

EFFECT OF DISSOLVED SALTS AND HUMIC ACID ON BREAKTHROUGH VOLUMES

Compound	Solvent	Breakthrough Volume, mL With salt	Without salt
Benzophenone	20% methanol	445,430	470,445
	30% methanol	155, 150, 148	164, 155
	40% methanol	55, 56, 55	61, 57, 54
	50% methanol	22, 22, 22	22, 22, 23
Caffeine	Water	345, 347	354, 333
Cinnamic acid	Water (pH 3)	15.5, 15.5, 15.8	15.8
Furfural	Water	—	18.5, 18.5, 18.5
	Water plus 10 ppm humic acid	18.6, 18.4, 18.45	18.8, 18.8, 18.65

Note: column was 0.45 cm x 10.5 cm containing 0.67 gram Bondapak-C_{18}

3b. Caffeine. Caffeine was chosen for detailed study because it is a common contaminant in wastewater, is sufficiently hydrophobic to be adsorbed by Bondapak-C_{18}, yet is freely soluble in water. Tests could therefore be made with purely aqueous solutions. Water does not wet Bondapak-C_{18}; this adsorbent must be treated with methanol (or other organic solvent) before it can remove organic compounds from water. Dilute methanol solutions are not good enough; it is best to condition the Bondapak with pure methanol and flush the methanol out before proceeding. The following breakthrough volumes were found with 5 ppm caffeine in simulated city water and a column of Bondapak 0.45 mm ID x 10.5 cm long:

Fresh Bondapak, dry-packed, no contact with methanol: V_{ads} = 2.5 ml
After passing 250 ml 20% methanol, then aqueous caffeine: V_{ads} = 55 ml
After passing another 250 ml 20% methanol: V_{ads} = 200 ml

Thereafter, 20% methanol (250 mL) and aqueous 5 ppm caffeine were passed alternately. Successive breakthrough volumes for the caffeine were as follows: 202, 199, 200, 194, 163 mL.

A second column of the same dimensions as the first, but packed separately, gave these retention volumes:

Conditioned with 20% methanol, then aqueous caffeine passed as before; successive breakthrough volumes, with intervening rinses with 250 ml 20% methanol, were: 171,214,193,209 mL.

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Then, successive portions of 5 ppm aqueous caffeine were passed with intervening rinses by water, not 20% methanol. Breakthrough volumes were: 186, 200, 182, 197, 172 mL. Then after passing 450 mL simulated city water (see above), breakthrough volumes for caffeine were: 6, 5.5, 6 mL.

Many tests like this were run, and we concluded that:

a) The column should be conditioned with methanol of at least 50% concentration;

b) A few mL of pure methanol brought the column to its maximum absorptive capacity; however,

c) No conditioning technique would make V_{ads} for aqueous solutions perfectly reproducible.

d) A column that had been conditioned with methanol, then repeatedly loaded and stripped with caffeine in aqueous solution, showed progressively decreasing V_{ads}. In part, the decrease was due to incomplete stripping of caffeine by water, because caffeine that remained on the column could be recovered afterwards by passing pure methanol. The last portions of adsorbed caffeine came out very slowly.

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Figure 3 shows adsorption-desorption curves for caffeine, 10 ppm in simulated city water, on a Bondapak column 0.45 cm ID x 5.5 cm long that had previously been conditioned with methanol. These curves are traced from records on the strip-chart recorder. Essentially the same curves were obtained with three different flow rates: 2.5, 5.0, 7.5 mL/min. The contrast between these curves and those shown in Fig. 2 is clear; we see a rapid concentration rise during breakthrough, a slower concentration fall during stripping. This behavior is due to a nonlinear adsorption isotherm; adsorption becomes less strong, that is, the distribution ratio falls, as the concentration rises; this is a common phenomenon in chromatography. Measurements of V_{ads} at different concentrations confirms this interpretation; see Table 4.

TABLE 4

ABSORPTION VOLUMES OF CAFFEINE
ON BONDAPAK-C_{18} FROM WATER

Caffeine concentration, ppm:		1	2.5	10
Absorption volume, mL, at flow rates:	2.5 ml/min	70	71	48
	5.0 mL/min	65	62	46
	9.9 mL/min	63	56	42

Column 0.45 cm x 10 cm, freshly packed.

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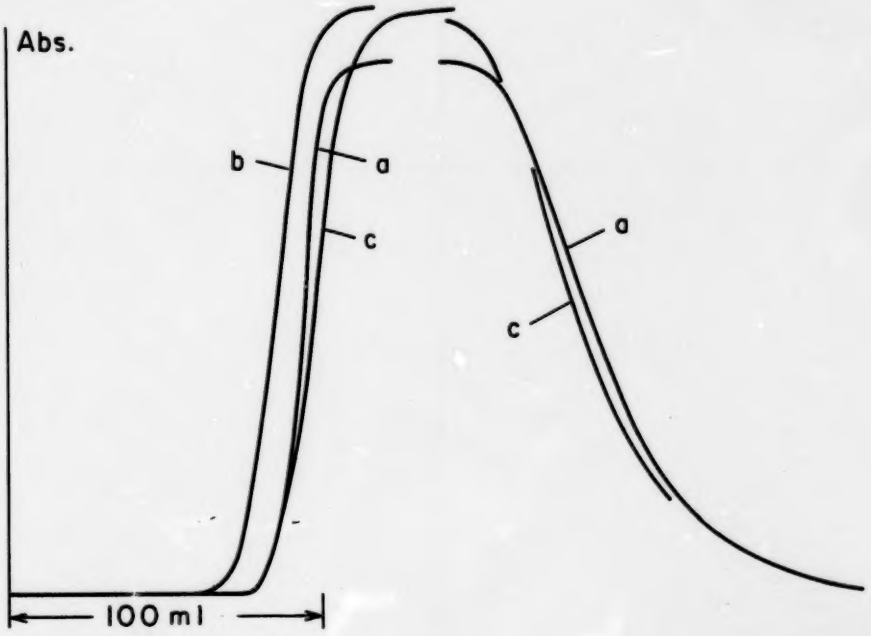

FIGURE 3. ADSORPTION AND DESORPTION OF CAFFEINE ON BONDAPAK-C_{18} FROM WATER

These curves are copied from strip-chart records. Caffeine concentration was 10 mg/L; flow rates, curve (a), 2.5 mL/min, 0.275 cm/sec.; (b), 5.0 mL/min, 0.55 cm/sec.; (c), 7.5 mL/min, 0.825 cm/sec. Column dimensions, 0.45 cm x 5.5 cm. Absorbance measured at 280 nm, 1.0 absorbance units full scale. Note the small dependence on flow rate and the unsymmetrical shape; a nonlinear adsorption isothern is indicated.

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In conclusion: Caffeine is adsorbed from water in an irreproducible manner that depends on the presence of small amounts of methanol in the absorbent. The adsorption isotherm is not linear, and a little of the caffeine, perhaps 2-5%, is retained very strongly by the absorbent, perhaps in deep pores or on active adsorption sites. It diffuses out slowly. Reproducible absorption-desorption behavior is only obtained in solvents containing at least 20-30% of an organic component like methanol, ethanol or acetonitrile. One must also bear in mind that it is difficult to pack columns reproducibly, and that the adsorbent may deteriorate on storage if the ambient fluid is not chosen properly.

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3c. Furfural. This is one of the Consensus Voluntary Reference Compounds (Keith et al, Environ. Sci. Tech., 13, 1469, 1979) recommended for evaluation by (HERL, EPA). Breakthrough volumes were measured at methanol concentrations ranging from 50% to 2%, and in pure water. Figure 4 shows the relationship. The logarithm of the breakthrough volume, corrected for the void volume of the column, is plotted against the volume fraction of methanol in the solvent. As a rule, such plots give a straight line, like the benzophenone plot in the figure, but there is evidence that the linear relationship breaks down at low methanol concentrations; adsorption on hydrophobic bonded-phase adsorbents, like Bondapak-C_{18}, is stronger in pure water and at very low methanol concentrations than the straight-line plot would predict. We found this to be true of caffeine also. It is commonly thought that in solvents rich in methanol, the long bonded hydrocarbon chains stretch out into the solvent and move freely about their anchor points on the silica surface, but in pure water they stick to each other to form a compact, highly hydrophobic, matted layer; see Figure 5. (Engelhardt and Ahr, Chromatographia, 14, 227, 1981; Scott and Simpson, J. Chromatogr. 197, 11, 1980).

The compound crotonaldehyde behaved like furfural, but was retained somewhat less strongly.

3d. Other compounds. Substances from the list of Consensus Voluntary Reference Compounds were passed through a column of Bondapak-C_{18}, 0.45 cm ID x 10.5 cm long, and elution volumes, V_{el}, measured. Most of these substances are sparingly soluble in water and very strongly retained; retention volumes were therefore measured in water-methanol mixtures. Each measurement was made in triplicate; the three values almost always agreed to within 2-3 percent; see, for example, the results for benzophenone, Table 2 and the data for 2,4-dichlorophenol shown in Table 5. The concentrations of the solutions used in breakthrough tests had to be high enough to be measured by ultraviolet absorbance; they ranged between 1 and 50 ppm. We verified that V_{ads} was independent of the concentration and can confidently assume that in the parts-per-billion concentrations found in wastewater, V_{ads} would be no less. For some compounds the linear relation between (log V_{ads}) and the volume fraction of methanol was verified by using three or more methanol concentrations ; for others, only two methanol concentrations were used. For compounds that are acidic (chloro- and nitrophenols) it is important to add acid to the solvent to repress the ionization. The pH value must be more than 3-4, however, or the hydrocarbon bonded phase is destroyed. We found that adding 0.1% of acetic acid to the solvent was sufficient to repress the ionization of the chlorophenols, including pentachlorophenol, and to assure reproducible behaviour.

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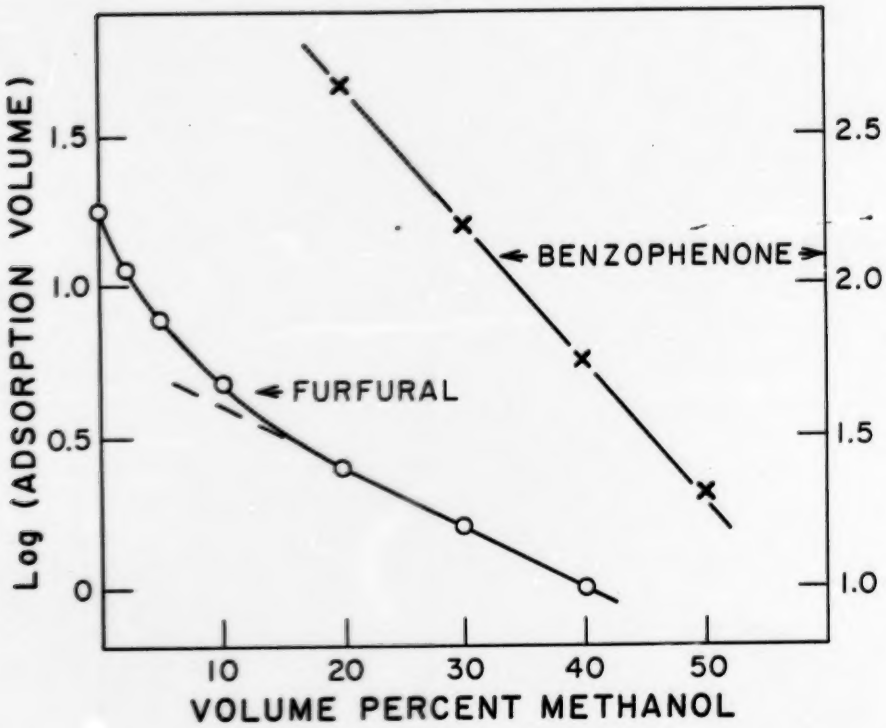

FIGURE 4. ADSORPTION (BREAKTHROUGH) OF FURFURAL AND BENZOPHENONE ON BONDAPAK-C_{18} VERSUS VOLUME FRACTION OF METHANOL

Furfural column was 0.45 cm x 10.5 cm, void volume 0.8 mL; benzophenone column was 0.32 cm x 11.6 cm, void volume 0.6 mL. Adsorption volumes are in mL, corrected for the void volume.

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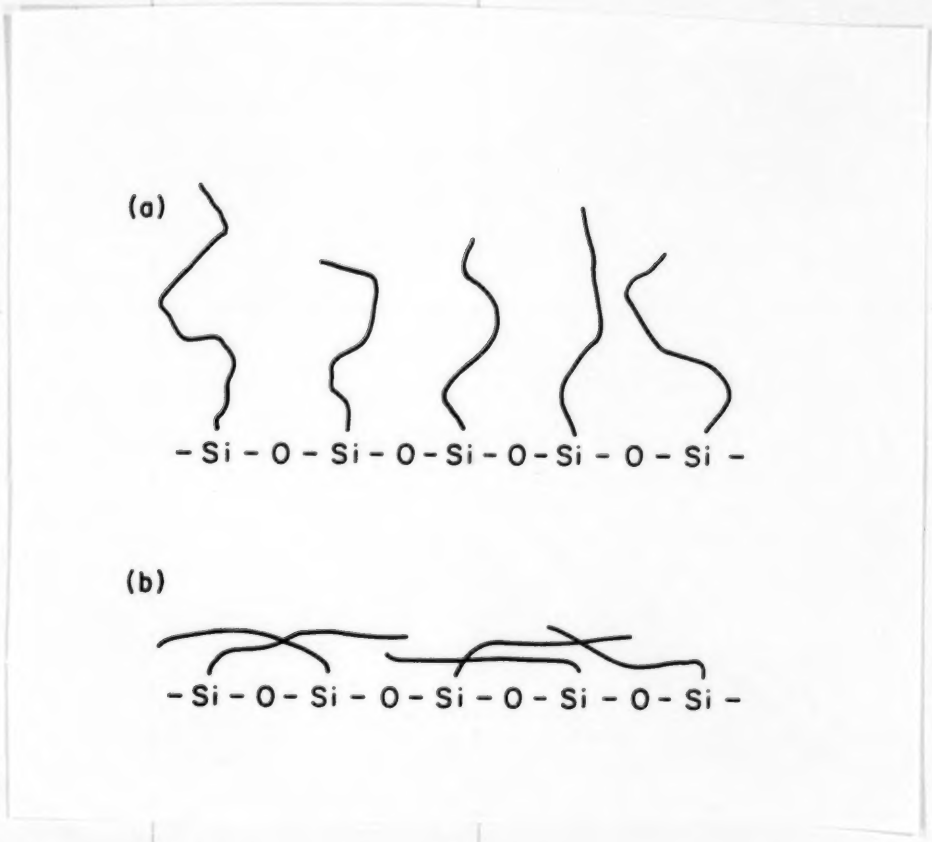

FIGURE 5. CARBON CHAINS ATTACHED TO SILICA SURFACE (HYPOTHETICAL); (a), in methanol; (b), in water. See R.P.W. Scott and C.F. Simpson, J. Chromatogr., 197, 11 (1980).

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TABLE 5

ELUTION VOLUMES OF 2,4-DICHLOROPHENOL ON BONDAPAK-C_{18}

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Solvent, volume percent methanol	Recorded elution volumes, mL	Mean elution vol. Gross	Mean elution vol. Corr.for void	Log (corrected volume)	Log k'
30	21.9, 21.9	21.9	21.3	1.33	1.55
40	9.6, 9.6, 9.6	9.6	9.0	0.95	1.17
50	5.4 (five) (readings)	5.4	4.8	0.68	0.90
60	3.00 (five readings) 2.85 (three readings)	2.95	2.35	0.37	0.59

Notes: (a) column dimensions, 0.45 cm x 10.5 cm
(b) 0.1% acetic acid was added to all solvents to repress ionization
(c) void volume, measured by injecting sulfosalicylic acid, was 0.60 ml (found in many measurements)
(d) k' is the capacity factor, the corrected elution volume divided by the void volume.

Plots of (log V_{ads}) against methanol concentration are shown in Figure 6. By extrapolating the straight-line graphs to zero methanol concentration one may estimate the breakthrough volumes in pure water, but the actual breakthrough volumes on methanol-wetted Bondapak-C_{18} are probably higher; see Fig. 4 and discussion in Sec. 4, Part 3c.

Many elution volumes, V_{el}, were measured in columns of microparticulate, high-resolution C_{18} bonded phases, like Whatman Partisil ODS. Chemically these materials are identical to Bondapak-C_{18}, and as far as we know, selectivity orders are the same. Knowledge of these elution volumes allows us to estimate retention efficiencies for trace organic compounds in water and also helps us to interpret complex chromatograms: see Part II of this report. We have published the results of one such study, where we measured elution volumes of substituted biphenyls and biphenylols at different methanol concentrations and at three temperatures.[1] We found that the linear relation between (log V_{el}) and volume fraction of methanol was closely obeyed. Chlorine atoms increased retention, while hydroxyl groups decreased retention to almost the same extent. Raising the temperature reduced retention; by comparing the elution volumes at three

1. A. Hernandez and H.F. Walton, "Liquid Chromatography of Chlorinated Biphenylols", J. Chromatogr., 242(1982), 346.

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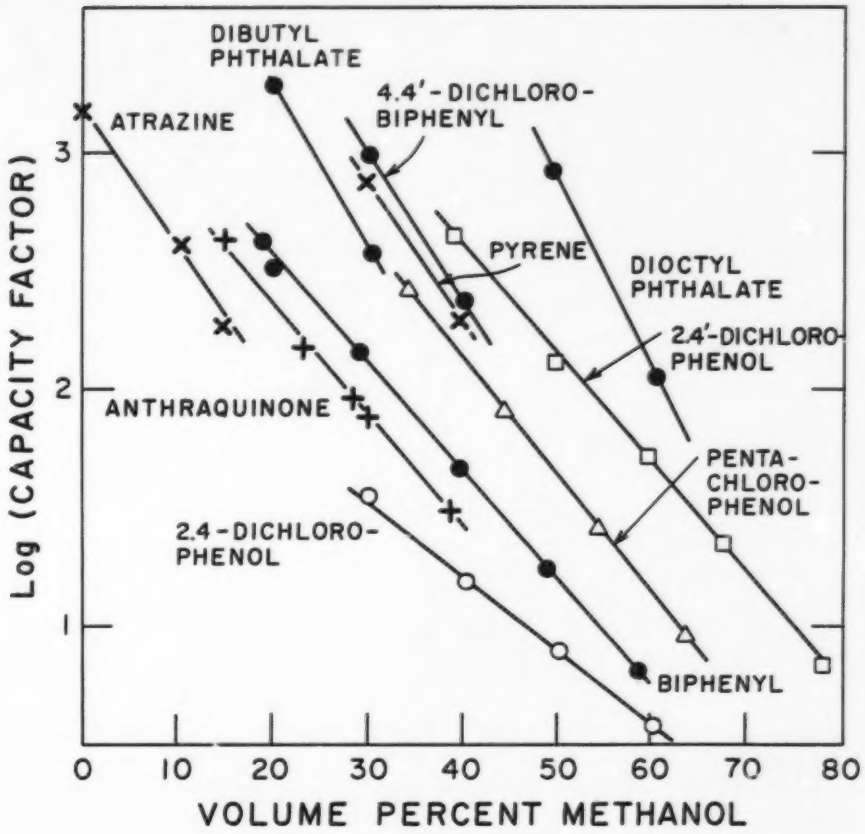

FIGURE 6. PLOTS OF CAPACITY FACTORS VS. METHANOL CONCENTRATION ON BONDAPAK-C_{18}

Column, 0.45 cm x 10.5 cm; void volume 0.6 mL; to find the adsorption volumes in mL, add (log 0.6 = -0.22) to ordinates.

temperatures we calculated heats of adsorption, and found them to range from 2 to 5 kcal/mole, being higher, the lower the methanol content.

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SECTION 5

LIQUID CHROMATOGRAPHIC ANALYSIS OF WASTEWATER

1. By large-scale trace enrichment and gradient elution.

This is the technique described in our 1979 report. Several liters of filtered wastewater are pumped through a column of Bondapak-C_{18}, 1 cm diameter x 50 cm long, and the absorbed materials are then stripped from the column by a water-to-methanol gradient. An ultraviolet absorbance detector is placed at the column exit. Five fractions are collected, designated G1 to G5 in order of decreasing polarity. Two of these are of special interest and were subjected to further fractionation: G2 and G5.

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1a. The green G2 fraction. When the water-to-methanol gradient is started, the first thing to happen is the emergence of a dark brown humic or fulvic fraction which we called G1. Then, sharply, the brown color changes to a green, and for several minutes the liquid flowing out of the column is an intense blue-green. Then, again sharply, the green changes to a brownish yellow. The green fraction was collected separately and called G2.

We subjected the G2 fraction to size-exclusion chromatography on a column of TSK Gel 2000 SW, a hydrophilic medium, based on silica gel, made by Toyo Soda Co., Japan. The solvent was 0.05 M sodium phosphate in 20% ethyl alcohol, pH 7.5. The first solution to come out of the column is yellow-brown. A blue color appeared near the end of the run at the low-molecular-weight range, below 1000 Daltons, perhaps below 500. By running several G2 samples in succession through the column and collecting and combining the blue fractions we could get enough of the blue material to study. Its UV-visible absorption spectrum was distinctive; there was a peak at 630 mm with a shoulder on the low-wavelength side, then no more absorption until well into the ultraviolet, below 300 mm. The spectrum was identical with that of the blue dye used in toilet bowl cleaners. The dye is probably a cynanine and is evidently very stable, since it survives secondary sewage-treatment.

The molecular weight range of the brown humic material in fraction G2 is 1,000-10,000, with a peak in UV absorbance at molecular weight 5,000. Fraction G1 was put through the same size-exclusion column and showed a molecular weight around 3,000. After treating G1 with chlorine and removing the excess chlorine the chromatogram showed a maximum at molecular weight of 2,000, and some small peaks of lower molecular weight appeared. Evidently, treatment with chlorine breaks down the molecules of humic acid.

We made a number of experiments with humic fractions on the TSK Gel 2000 SW column, and noted that the apparent molecular weight, as indicated by the elution volume, depends on pH; it increases as the pH is raised. This fact indicates that the humic acids are linear polymers that coil up and are compact at low pH, whenthe acid

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is not ionized, and uncoil and become extended at high pH, when the molecules are ionized and carry negative charges that repel each other. Tests were made up to pH 9, the highest pH that a silica packing can tolerate without dissolving, and showed clear evidence of two stages of ionization; one around pH 5, corresponding to ionization of carboxyl groups, and another near pH 9, corresponding to phenolic hydroxyls.

1b. The least polar fraction. The last fraction we collected in gradient fractionation, called G5 in our earlier report, contains many ultraviolet-absorbing compounds, as shown by high-resolution liquid chromatography. The fraction before it, G4, contains compounds that give distinct peaks in these columns, but most of the organic material in G4 seems to be humic. The G5 fraction is collected from the time that the solvent program reaches 100% methanol, that is, the time that pure methanol begins to enter the 50-cm Bondapak column. As collected, it is almost colorless, but after evaporation to small volume it is faintly yellow or pink.

A "pooled" G5 concentrate of volume 5 mL was prepared by evaporating the fractions from several runs; it represented some 50 liters of wastewater. Samples of 50-100 microliters were injected into high-resolution microparticulate bonded C_{18} columns with various methanol-water mixtures. The best concentration was 70% methanol. The variable-wavelength Schoeffel detector was used in conjunction with the Schoeffel fluorescence detector or the BAS electrochemical detector. Some spectacular chromatograms were obtained with many closely-spaced peaks in the ultraviolet; fluorescence gave fewer peaks which came in different places; electrochemical peaks were still fewer and occurred with the smaller elution volumes. The electrochemically active compounds were probably phenols, perhaps benzidines; the fluorescent compounds might have been phenol ethers. The problem of peak identification in such complex mixtures is almost insuperable. A further difficulty was that the peak pattern changed with time. Repeated injections on the same day gave similar chromatograms, but a week or a month later, a number of the peaks had disappeared. The concentrate deteriorated on storage, even under refrigeration.

Typical chromatograms are shown in Figures 7 and 8.

2. Evaluation of Denver pilot plant for tertiary treatment.

Though it may be hard to identify individual peaks in the chromatograms, one may use liquid chromatography combined with trace enrichment to evaluate treatment processes. In the summer of 1980 the Denver Water Board operated a small-scale pilot plant to test procedures for water reuse. The secondary effluent was treated with alum and lime, then passed through a bed of clinoptiolite, followed by granular activated carbon. Then it was treated with ozone. Following ozone treatment, the water was passed through a second bed of granular activated carbon.

We took samples at four points:
a) after clinoptiolite treatment;
b) after the first carbon bed, before ozone;
c) after ozone treatment;
d) after the final carbon treatment.

We measured dissolved organic carbon and found 5.6, 2.4, 2.1 and less than 1.0 ppm, respectively. (The limit of detection was 0.5 - 1.0 ppm C). Also we examined each sample by trace-enrichment liquid chromatography on a high-resolution C_{18} column,

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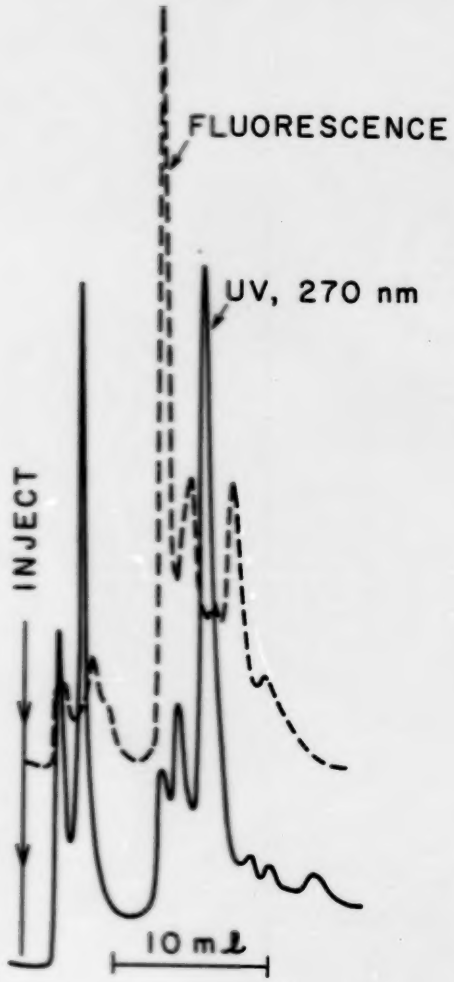

FIGURE 7. CHROMATOGRAM OF POOLED G5 WASTEWATER FRACTION; SEE TEXT

Column was microparticulate C_{18}-silica (Whatman); eluent was 70% methanol (isocratic); full scale absorbance, 0.1 unit. Fluorescence was excited at 270 nm.

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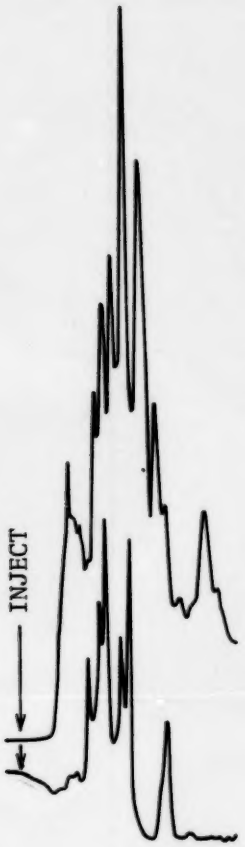

FIGURE 8. CHROMATOGRAM OF ANOTHER G5 (END-OF-GRADIENT) WASTEWATER FRACTION; SEE TEXT

Column was microparticulate C_{18}-silica (Whatman); eluent was 70% methanol containing 0.10M ammonium acetate. Upper curve UV absorbance at 270 nm (0.1 unit full scale); lower curve shows electrochemical current at 0.8 volt vs. saturated calomel electrode.

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Whatman PAC-ODS, 0.45 cm ID x 25 cm long, fitted with a Brownlee precolumn, 0.45 cm x 4 cm. Portions of 100 ml were pumped through the column at 1.0 mL/min, followed by pure water for 15 min, then a linear water-to-methanol gradient for 30 min, then pure methanol for 20 min. In addition, a blank was run with purest distilled water that had been irradiated with ultraviolet light to destroy organic matter. The curves of UV absorption (270 nm) vs. time are shown in Figures 9, 10 and 11. Note the lower detector sensitivity in Figure 9.

The final product from the pilot plant was nearly as pure as distilled water and much purer than city drinking water. It seems that the ozone treatment does not destroy organic carbon, but rather breaks the molecules into fragments that can be absorbed by granular activated carbon.

3. Analysis by steam distillation, followed by liquid chromatography.

Many organic compounds that are not sufficiently volatile to be recovered by the purge-and-trap method can nevertheless be removed from water by steam distillation. The water is boiled under reflux for several hours in an apparatus that makes the condensate pass through a layer of immiscible nonaqueous solvent, either heavier than water like methylene chloride, or lighter than water like hexane. We used both types of apparatus, the first homemade, the second a product of Kontes Glass Company (Cat. No. K-523025).

The solvent containing the captured trace organic compounds was evaporated just to dryness at room temperature, and the residue was taken up in 0.1 mL of pure methanol. The methanol solution was injected into a Whatman PXS-ODS analytical column like that described in the last section; the solvent was 70% methanol; UV and fluorescence detectors were used in series. The chromatograms obtained contained a few strong peaks, and with some effort these peaks might have been identified. Different peak patterns were obtained from wastewater at its natural pH (6.7, rising to 9.0 as carbon dioxide was lost during boiling) and from wastewater that was first acidified to pH 3. Carboxylic acids would be vaporized at pH 3, but retained as their salts at pH 7-9. Chromatography with an electrochemical detector revealed three peaks in the acid distillate; these were probably phenols.

Gas chromatography-mass spectrometry on the methylene chloride extract obtained by refluxing an acidified solution showed the presence of fatty acids. C_nH_{2n+1} COOH, with n=13, 14 and 15, also the methyl ester, $C_{15}H_{31}COOCH_3$.

We did not spend much time on steam distillation, but it is a promising technique that would reveal a limited group of compounds (fatty acids and esters, polychlorinated biphenyls, polycyclic aromatic hydrocarbons) and perhaps be of diagnostic value.

4. Small-scale trace enrichment.

In our 1979 report, and in Part II.1 of this report, we described a trace enrichment technique that uses a large Bondapak column and ten or more liters of wastewater. The drawbacks to this technique are obvious: it is cumbersome and takes a long time, unstable compounds may decompose or be oxidized during this time, and the adsorptive capacity of the Bondapak-C_{18} probably diminishes during the long pumping period. We have therefore experimented with a small-scale analytical technique that uses a disposable "Sep-Pak" (Waters Associates) for preconcentration. The "Sep-Pak" is a small plastic

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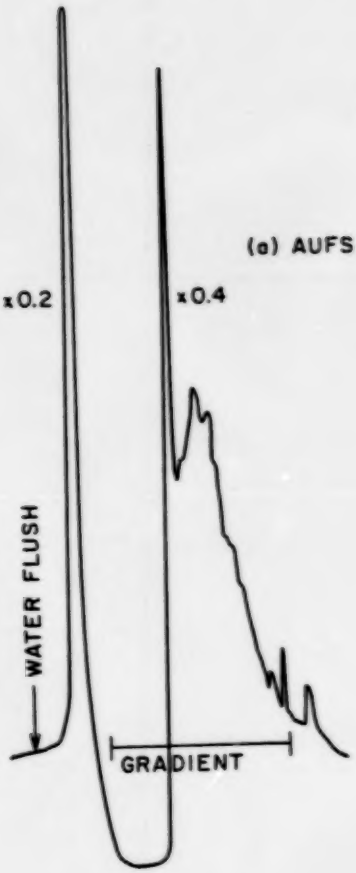

FIGURE 9. CHROMATOGRAM OF 100 ML of DENVER SEWAGE EFFLUENT FOLLOWING CLINOPTIOLITE TREATMENT; SEE TEXT.

(Copied for strip-chart record). Total organic carbon = 5.6 ppm.

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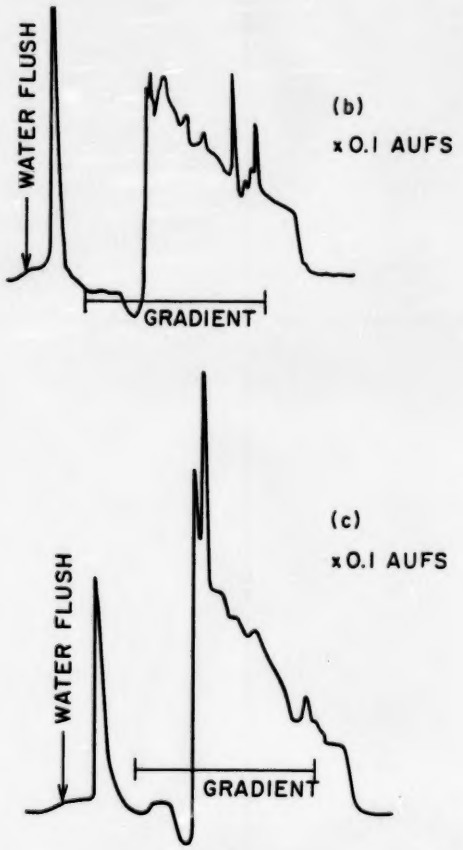

FIGURE 10. CHROMATOGRAM OF 100 mL DENVER SEWAGE EFFLUENT, (b) AFTER FIRST CARBON COLUMN IN TERITARY-TREATMENT PILOT PLANT; TOTAL ORGANIC CARBON = 2.4 ppm; (c) AFTER OZONE TREATMENT IN PILOT PLANT; TOTAL ORGANIC CARBON = 2.1 ppm; SEE TEXT

UV absorbance at 270 nm is shown; note full-scale absorbances and compare with Figure 9.

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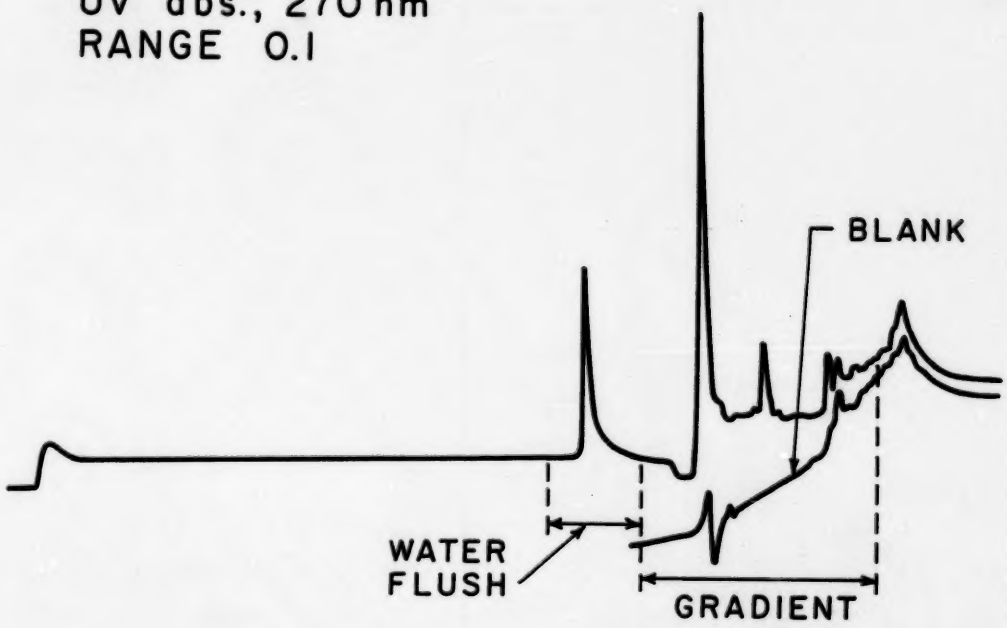

FIGURE 11. CHROMATOGRAM OF 100 mL FINAL EFFLUENT FROM DENVER PILOT PLANT, FOLLOWING THE SECOND CARBON COLUMN; TOTAL ORGANIC CARBON, 1.0 ppm; SEE TEXT.

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cylinder, 1 cm ID x 1 cm long, packed with Micro Bondapak-C_{18}, with particles 10 microns in diameter. This is the same adsorbent that is used in high-resolution analytical columns. We emphasize that the method we are describing is intended for analytical purposes, not to prepare fractions for toxicity testing.

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A serious difficulty in analyzing the potentially toxic trace organic compounds in treated sewage is the presence of humic material. Humic material is not a single chemical substance, but an ill-defined mixture of high molecular weight with molecules that carry a variety of chemical functional groups. It is extremely difficult to separate the humic material from the other substances present in wastewater. In our previous technique, some humic material persisted in our least polar G5 fractions and caused a smearing of the chromatograms. We have not solved the humic problem with our new technique, but we think we are approaching a solution.

If secondary-treated sewage is forced through a C_{18} "Sep-Pak" that has been previously conditioned with methanol, the humic material is held back as a thin brown ring at the entrance to the Sep-Pak, and the water that flows out of the Sep-Pak is clear and colorless. Later we strip the Sep-Pak with methylene chloride, which removes the organic compounds of interest, and leaves nearly all the humic material behind. No effort is made to remove the humic material from the Sep-Pak; this item is discarded after use.

The apparatus used is the following: a glass syringe of 50 or 100 ml capacity; a Sage syringe pump; a liquid chromatography assembly consisting of two Waters Model 6000A pumps, Model 660 solvent programmer, U6K injector, a Valco six-port valve, a Whatman PXS-ODS analytical column and a Brownlee precolumn, a Schoeffel variable-wavelength ultraviolet detector and a Schoeffel fluorescence detector with a Linear Instruments two-pen recorder. The valving system is shown in Figure 12. Its purpose is to allow one to inject a fairly large sample, up to 2 ml, which is the volume of the storage loop of the U6K injector, and collect the dissolved compounds on the Brownlee precolumn; then, by passing a higher concentration of methanol and reversing the solvent flow, to focus the various dissolved compounds so that they enter the analytical column together in a small volume.

The procedure is as follows:

a) Sample preparation. Filter a quantitly of secondary sewage effluent through Whatman glass-fiber filter paper, grade GF/F; adjust the acidity with nitric acid if desired. Prepare a C_{18} Sep-Pak by passing 2 ml methylene chloride followed by 5 ml methanol. Fill a 100-ml syringe with the filtered wastewater, attach the Sep-Pak to the Luer tip of the syringe, place the syringe in the Sage pumping assembly, and drive the filtered wastewater through the Sep-Pak at 5 mL/min. The volume driven through the Sep-Pak may range from 50 to 200 ml, depending on the expected concentration of organic compounds.

Now force about 50 mL of air downwards through the Sep-Pak to remove as much water as possible. Then pass, slowly, 3-4 ml methylene chloride, collecting the solvent in a vial. A little water accompanies the solvent; remove the water by blotting with a torn strip of filter paper. Evaporate the methylene chloride just to dryness in a stream of nitrogen.

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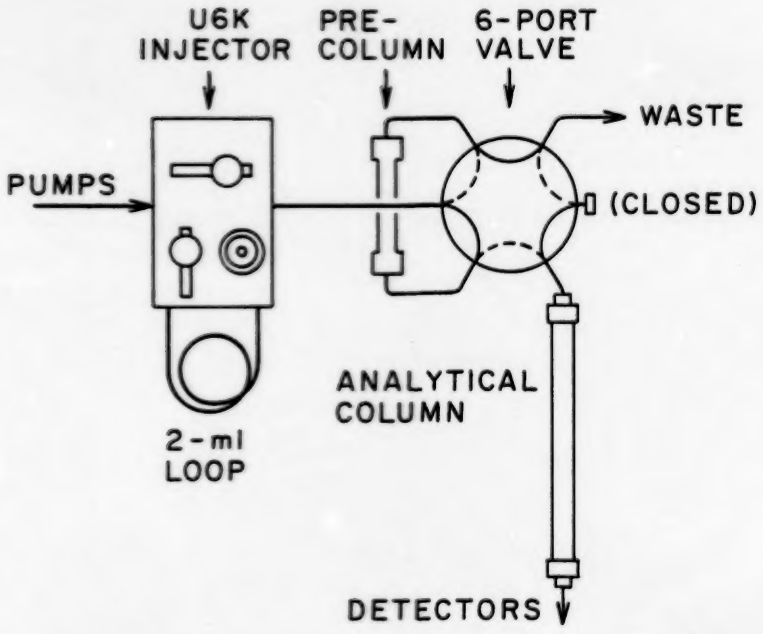

FIGURE 12. VALVING SYSTEM FOR SECOND STAGE OF TRACE ENRICHMENT AND ANALYTICAL HIGH-RESOLUTION CHROMATOGRAPHY.

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Take up the residue in 0.1 - 0.15 ml methanol, and add 1 ml water. The solution is pale yellow and may be slightly cloudy.

b) Sample introduction; chromatography. The first Waters pump is fed with two solvents, 10% methanol and 25% methanol; the inlet valve allows one to select either solvent. The second pump is fed with 75% methanol. Before introducing the prepared sample, pump 10% methanol through the entire system at 1.0 mL/min.

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Introduce part or all the prepared sample into the Waters U6K injector. Let us suppose we introduce half of it, 0.5 mL. Have the U6K valve in the LOAD position, the Valco valve in LOAD position (shown by solid lines in figure). Turn the Waters valve to INJECT and wait 45 seconds. The sample is now transferred to the precolumn.

Now turn the Valco valve to the INJECT position (broken lines in Fig. 12). Flow through the precolumn is now reversed, and the absorbed compounds are brought back to the point of entrance. The weakly absorbed, fast-moving compounds travelled farther down the precolumn than the strongly absorbed, slow-moving compounds, and they move back faster, causing a focusing effect.

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Wait 45 seconds (the same time that was used to load the precolumn), then do three things:

Switch the inlet valve of the first Waters pump to admit 25% methanol;

9-1/8"

Start the solvent programmer to give a linear gradient, 25% to 75% methanol in 30 minutes;

Mark the recorder paper to show the start of run.

Run through the gradient and keep going until the recorder pen returns to baseline.

Recorder traces for a Denver wastewater and for two standard mixtures are shown in Figures 13 and 14. They were recorded by UV absorption at 265 nm. Figure 15 shows the fluorescence record for a Denver and a Boulder wastewater. We note the prominent peaks in the Boulder product that are not present in Denver wastewater. An industrial plant that manufactures fine chemicals contributes prominently to the Boulder wastewater.

The efficacy of the reverse-flow focusing system was tested several times with mixtures of pure compounds. In one such test, 50 microliters of a solution of caffeine, t-butoxyphenol, 9-fluorenone and dibutyl phthalate was diluted to 2 ml with 10% methanol and introduced in the manner described. Instead of using a gradient, however, the methanol concentration was raised to 60% and held constant at this value. The recorder trace is shown in Figure 16. The peaks are even sharper than those obtained by direct injection.

The UV chromatograms are complex, but they are reproducible. Portions of the same filtered wastewater passed through different Sep-Paks and worked up at different times gave essentially identical chromatograms. The methanol extract, however, is not stable. If half of it is injected on one day and the other half three days later, the second chromatogram has fewer peaks and the ratio of peak heights is changed. One

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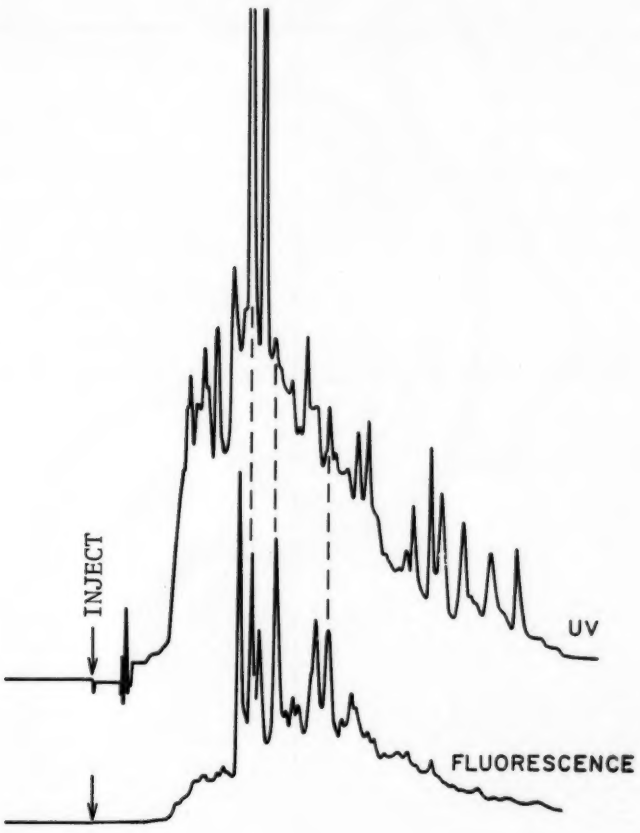

FIGURE 13. SIMULTANEOUS UV (265 nm) AND FLUORESCENCE CHROMATOGRAMS FOR 60 mL of DENVER SECONDARY SEWAGE EFFLUENT, USING SYSTEM SHOWN IN FIG. 12 AND DESCRIBED IN TEXT. UV ABSORBANCE IS 0.1 UNIT FULL SCALE.

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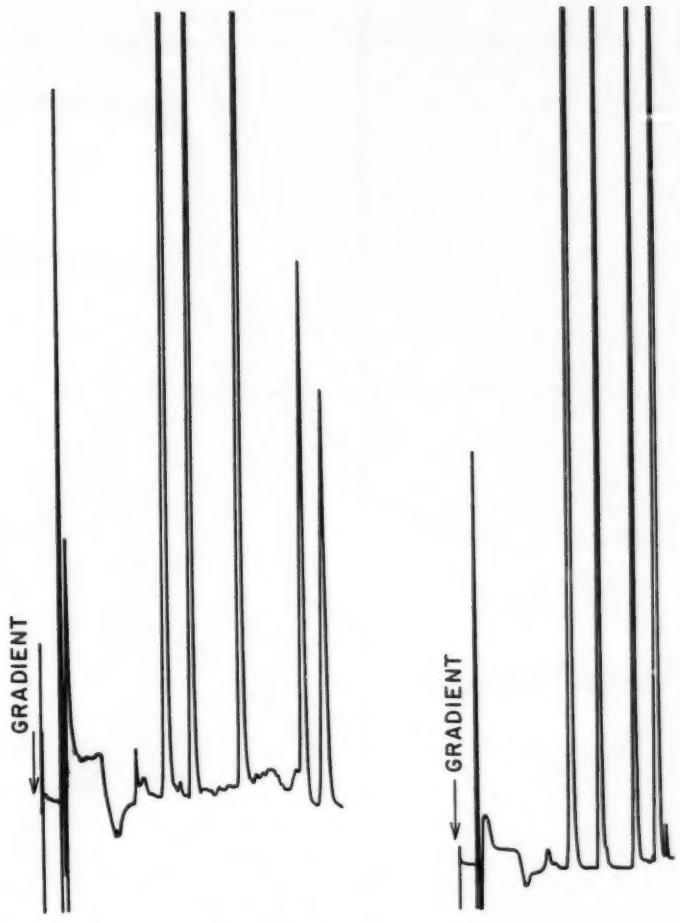

FIGURE 14. UV CHROMATOGRAMS OF SYNTHETIC MIXTURES, USING SYSTEM SHOWN IN FIG. 12.

Detection wavelength, 274 nm; 0.1 absorbance unit full scale. First mixture, phthalate esters — dimethyl, diethyl, dibutyl, benzyl butyl and dioctyl; second mixture, caffeine, t-butoxyph nol, 9-fluorenone, dibutyl phthalate. Quantities injected ranged from 2 to 20 micrograms.

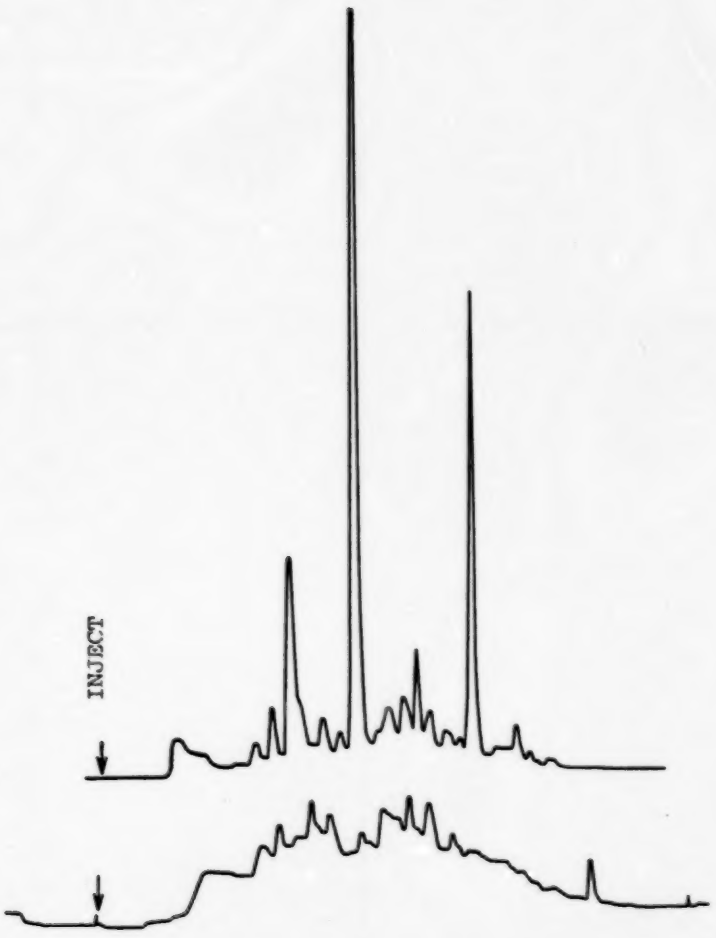

FIGURE 15. FLUORESCENCE CHROMATOGRAMS OF TWO SECONDARY SEWAGE EFFLUENTS; (a) BOULDER, (b) DENVER.

Each injection correspond to 10 mL of secondary effluent. Solvent gradient went from 25% to 75% methanol in 40 minutes.

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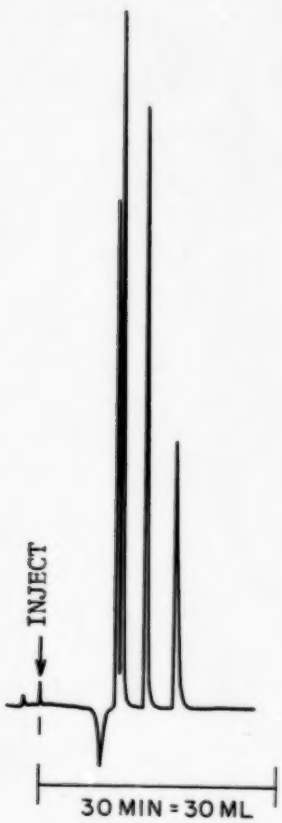

FIGURE 16. TEST OF REVERSE-FLOW FOCUSING.

2.0 mL of 20% methanol, containing 2 micrograms caffeine, 8 micrograms t-butoxyphenol, 10 micrograms 9-fluorenone and 20 micrograms n-butyl phthalate, were injected into the precolumn of Fig. 12 and backflushed into the analytical column with 60% methanol at 1.0 mL/min. Chromatogram was recorded at 280 nm with 0.2 absorbance unit full scale. Components were eluted in order named above; elution was complete in 20 minutes. Note sharp resolution of caffeine and t-butoxyphenol.

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must, therefore, run the chromatograms as soon as possible after extraction from the Sep-Pak. The whole operation, starting with filtered wastewater, takes about two hours:

Pass 100 ml wastewater through Sep-Pak:	30 min.
Extract Sep-Pak with methylene chloride, remove water, evaporate:	30 min.
Run the chromatogram with 30-min gradient:	60 min.

Identification of peaks in the chromatogram is difficult because there are so many of them. The retention volumes can be matched against those of standards, but this is only the beginning. Positive peak identification requires the use of selective detectors, including the new diode-array UV scanning detector, and perhaps the liquid chromatography-mass spectrometry interface. Retention volumes are unsatisfactory for peak identification on reverse-phase C_{18} columns because they depend so strongly on the number of carbon atoms; see the spread of retention volumes for the phthalate esters in Fig. 14. It would be more useful to identify compound classes or functional groups.

We have only just begun to study small-scale trace enrichment as an analytical method. The utility of such an analytical tool is obvious, especially in water reuse, and we hope to develop it.

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APPENDIX I

Adsorption-desorption curves for benzophenone

Benzophenone is a "model compound" whose adsorption from methanol-water mixtures seems to be reversible and reproducible. To test this statement and to provide more information, a steel column, 0.45 cm internal diameter and 15.0 cm long, was freshly packed with Bondapak-C_{18} from the batch received in May 1981. First, the empty column was connected to a shorter column of the same diameter, 10 cm long; the two columns were then dry-packed together, the packing being dropped through the short column and into the longer one, both being vibrated. Dry packing was preferred to slurry packing because this adsorbent has a wide range of particle sizes, and we wanted the packing to be as uniform as possible along the length of the column; slurry packing would inevitably have introduced some size segregation. Following dry packing, 90% methanol was pumped through the connected columns at the fastest speed provided by the pump, namely 9.9 mL/min; it entered through the shorter column. Then the two columns were disconnnected; the packing had subsided some 2 mm at the inlet to the shorter column; the longer column was full. The longer (15-cm) column was capped with an end fitting and frit, while the shorter column was emptied; the packing from the shorter column was collected, dried and weighed; from the weight of Bondapak in the shorter column, the weight of Bondapak in the longer column was calculated; it was 0.97 gram.

Two solvent reservoirs were filled, one with 40% methanol (v/v), the other with a solution of benzophenone, 8 mg/L in 40% methanol. They were connnected to the inlets of a Waters Model 6000A pump. The 15-cm Bondapak column was connnected to the pump exit; at the column exit was a Schoeffel Model SF770 variable wavelength ultraviolet absorbance detector, set at 252 nm, the wavelength of maximum absorbance of benzophenone. The pump was run at 1.00 mL/min while the absorbance was continuously monitored. First 40% methanol was passed, then the inlet valve was turned to pass the solution of benzophenone; at this point a mark was made on the recorder chart. Recording was continued until the absorbance had risen to a maximum and leveled off. The effluent was collected in a graduated cylinder to check the flow rate. The inlet valve was turned to admit pure 40% methanol, and a desorption curve was traced. The adsorption and desorption curves are reproduced in Figure 17. They are ilke the schematic curves shown in Figure 2.

Additional experiments were made to determine the "dead volume" of the pump, between the inlet valve and the pump outlet; this volume was found to be 3.0 mL. Subtracting this volume from the volume passed to reach half the maximum absorbance, the "adsorption volume", here represented at V_R', was found.

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The dashed lines of Figure 17 are curves calculated from the continuous-flow plate theory of Glueckauf (Trans. Faraday Soc., 51 (1955), p. 34; see also H. Purnell, "Gas Chromatography", Chap. 7: New York, John Wiley, 1962). The equation is:

$$\frac{C}{C^o} = \frac{1}{2} - A_e\left[\left(\frac{N}{VV_R'}\right)\frac{1}{2}(V_R' - V)\right]$$

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where:

C is the concentration of the solute, benzophenone, when a volume V has passed;

C^o is the concentration of solute entering the column;

V_R' is the volume at which $C = \frac{1}{2}C^o$;

N is the number of theoretical plates;

A_e means the error function.

To evaluate N, the volume V was measured at the point where $C = 0.1587C^o$. At this point the quantity in square brackets is unity, because the error funtion of unity is (0.5 - 0.1587) or 0.3413.

The dashed (calculated) curves of Figure 17 are displaced 5 mm, or 2.9 mL, to the right for clarity. Shifted back to the left, they fit the experimentally recorded curves almost perfectly. We may thus be confident that the adsorption-desorption process in 40% methanol is reversible and that a linear isotherm is obeyed. The number of theoretical plates, N, was 185; the plate height is thus 0.81 mm. The volume V_R' was 66.5 mL on adsorption, 68.0 mL on desorption, 68.5 mL on readsorption.

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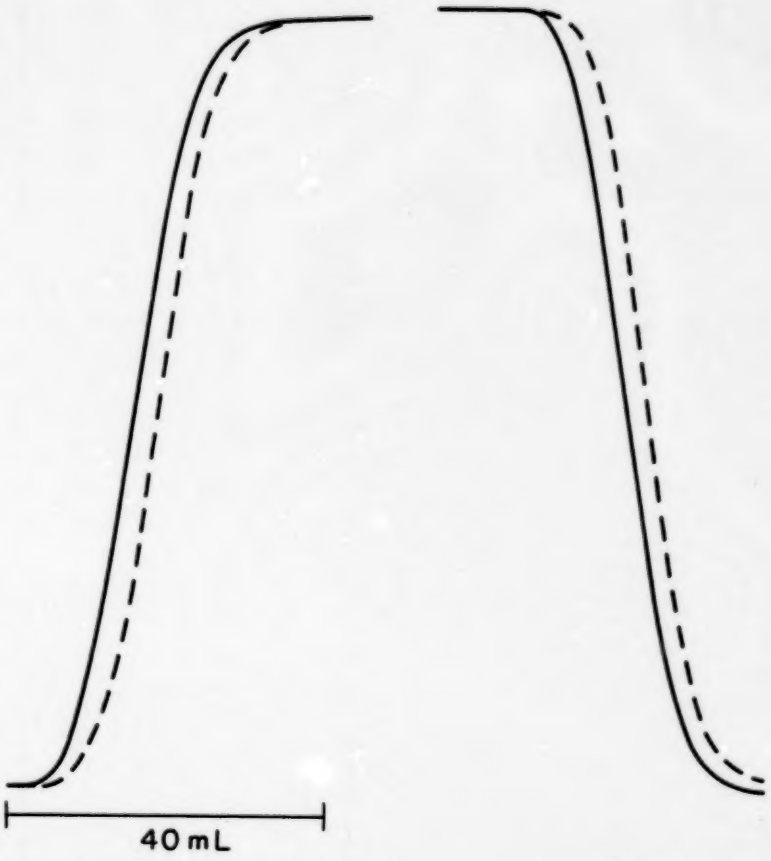

FIGURE 17. TEST OF REVERSIBILITY OF ADSORPTION OF BENZOPHENONE.

Column, 4.5 cm x 15 cm, packed freshly woth Bondapak-C_{18}; absorbance maimum 0.65 unit, wavelength 252 nm; solvent, 40% methanol; flow rate, 1.0 mL/min; for other conditions see Appendix I. Dashed curves are theoretical, solid curves experimental.